Proceedings of the 6th International Weber Symposium on Innovative Fluorescence Methodologies in Biochemistry and Medicine, Kauai 22-28 July 2005

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FLUORESCENCE-BASED SCREENING OF MICROBIAL STATUS DURING BIOPROCESSES.

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During microbial fermentations and other bioprocesses, monitoring of potential contamination, cell viability, physiological status and performance is of prime importance. As a given bioprocess advances, changes in population dynamics, cell physiology or process constituents will likely have impacts that require intervention. Various off-line procedures have been employed to estimate status of cells, for example identity, viability, stress tolerance and vitality. However the current techniques are both protracted and labour-intensive, so are not useful for monitoring rapidly changing processes. We are developing fluorescence-based techniques to provide feedback on cell status in rapid timeframes that enable realistic on-line control of process parameters. Spectroscopic approaches enable on-line analysis of cell populations using simple and robust instrumentation. While microscopy-based approaches remain labour intensive and must be carried out off-line, they provide important information on differential individual cell responses and spatial relationships that may be critical to overall performance. In parallel studies fluorescence-based approaches have been established or are under development to determine viability and identity, respectively. The major focus of investigations described here is assessment in whole cell systems of modulation of cell membrane fluidity, as an indicator of adaptability and vitality. Membrane fluidity is determined by fluorescence Polarization and Generalized Polarization of the membrane-localising probe 6-lauroyl-2-dimethylamino naphthalene (laurdan). Fluidity modulation has been observed in relation to physiological state (growth phase and glucose repression), temperature up shift and increased concentrations of ethanol. Furthermore, we detected effects on cell membranes of process additives that may potentially be toxic or membrane-active, in both yeast and bacterial systems.